ARMY MEDICAL SERVICE GRADUATE SCHOOL
WALTER REED ARMY MEDICAL CENTER
WASHINGTON 12, D. C.

IN REPLY REFER TO MEDEC-ZIBP

27 March 1953

Professor Joshua Lederberg
Department of Genetics
College of Agriculture
University of Wisconsin
Madison 6, Wisconsin

Dear Dr. Lederberg:

Your letter to Larry Weed was forwarded to this Division (Immunology) and the cultures you requested are being sent by Mr. Abrams who has charge of our culture collection.

At present, the Department of Bacterial Physiology of this Division is engaged in a project dealing with some phases of the problem of immunity to typhoid fever. Prior to my arrival here, I spent a short time at Cold Spring Harbor while receiving my degree with Professor Sol Spiegelman at Illinois, and as a result have been devoting the majority of time recently to studies of a genetic nature with various strains of typhoid.

I was fortunate enough to spend some time with Dr. Zinder during the last S.A.B. meeting discussing your recent paper as well as a few of the problems we were then starting to investigate. Since then we have made some slight progress, and after your letter arrived I discussed the situation with Dr. Weed and he suggested that I write, outlining the few results we have obtained. I am therefore enclosing a synopsis of our general approach and would very much appreciate hearing your opinion of our preliminary attempts.

If at all possible in the near future, I would more than welcome an opportunity to visit you at your laboratory for a few days, providing, of course, it could be arranged at your convenience. It is likely that any preparations necessary for my making a trip from here would probably require two weeks notice on my part. Aside from this detail, I will be very happy to make any arrangements suitable to your wishes. In the meanwhile, if there are any cultures or other material with which we can furnish you, we shall be more than happy to do so.

Sincerely yours,

Louis S. Baron

Louis S. Baron, Ph.D.
Department of Bacterial Physiology
Immunology Division

P. S. As an afterthought, I might mention that I was a student at Stuyvesant High School from 1937-1940.

AN ATTEMPT AT THE USE OF PHAGE LYSATES IN GENETIC TRANSFER

Our major interest lies in the fact that although all typhoid strains which contain the Vi antigen are not necessarily virulent for mice, no strain which lacks this factor is virulent for mice. In addition, all strains isolated from human cases of typhoid contain this antigen on isolation. With this situation in mind, we have been attempting to uncover a system which would cause transfer of this antigenic component as well as other more observable factors, namely carbohydrate fermentation and drug resistant, etc., between both Vi and non-Vi strains of typhoid and related organisms.

Since we have been actively engaged in this problem for only a few months, being primarily occupied in setting up basic techniques in an attempt to overcome a number of difficulties which we have encountered, our findings from the point of view of our original objective are as yet somewhat meager. However, I should like to mention some of the encouraging results obtained.

Our basic procedure has been as follows:

Donor Strain. Salmonella typhosa strain Ty2 possessing the antigenic composition Vi, IX, XII:, d xylose positive, streptomycin resistant, can be lysed by its specific Vi phage known as phage E_1. (This strain can be lysed by some of the Vi group phages which also lyse other Vi strains, however, phage E_1 fails to lyse any receptor strains.

Receptor Culture. Salmonella typhosa strain 643 possesses the identical antigenic structure as strain Ty2 but is streptomycin sensitive and xylose negative; this strain is lysed by the Vi group phages but not by Vi phage E_1.

A lysate of the donor culture is prepared in nutrient broth, phage E_1 on strain Ty2, X^+, S^r and filtered through a UF sintered glass filter. The filtrate is checked for sterility and assayed for phage count (usually about 4 x 10^8 phage particles/ml). The receptor culture, strain 643 X^-, S^s is then incubated with the phage filtrate from the donor strain, with nutrient broth as well as boiled filtrate as controls. After a short incubation period, the suspensions are washed and taken up in a few cc of saline and assayed for count/ml, usually 10^{10} cells. These suspensions are then plated on EMB xylose plates and nutrient agar plates overlayed with streptomycin agar. About 10^9 cells plated on EMB usually give rise to approximately 100-200 positive cells which may be related to the number of phage particles in the filtrate used to treat the cells. No positives are observed on the control plates although uninhibited negatives appear on both control and experimental plates. Similar results have been obtained with the transfer of streptomycin resistance, although the results have not been entirely consistant probably due to technical difficulties.

We have also performed a number of absorption experiments and it appears that certain Vi strains will absorb Vi phage E_1 rather well without resulting lysis taking place. Such is the case with strain 643 in our xylose and streptomycin resistance transfer experiments. However, as yet we have been unable to show any lysogenicity in either phage treated, phage absorbed or control strains, again possibly due to technical difficulties.

At the moment, we are engaged in antigen transfer experiments using these phage lysates on non-motile Vi strains and non-Vi strains of typhoid. Using semi-solid agar plates, we have noted the presence of spontaneously

motile variants in the controls of supposedly non-motile cultures. However, we are in the process of preparing lysates of other unrelated Vi strains such as S. paratyphi C, S. hallerup and E. coli 5396/38 using both Vi and O phages in an attempt to transduce unrelated flagellar antigens (other than the "d" factor) to our typhoid strains as was demonstrated in your recent paper. We have also been giving some thought to the transfer of the Vi antigen itself to non-virulent strains such as S. typhosa O-901 and have come to the conclusion that perhaps the mouse would be the best selective agent assuming that any transduced cell would become virulent.